Optimization of 1*H*-indazol-3-amine derivatives as potent Fibroblast Growth Factor Receptor inhibitors

Jing Cui, Xia Peng, Dingding Gao, Yang Dai, Jing Ai, Yingxia Li

PII: S0960-894X(17)30669-8
DOI: http://dx.doi.org/10.1016/j.bmcl.2017.06.068
Reference: BMCL 25102

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 2 March 2017
Revised Date: 31 May 2017
Accepted Date: 26 June 2017

Please cite this article as: Cui, J., Peng, X., Gao, D., Dai, Y., Ai, J., Li, Y., Optimization of 1*H*-indazol-3-amine derivatives as potent Fibroblast Growth Factor Receptor inhibitors, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: http://dx.doi.org/10.1016/j.bmcl.2017.06.068

Optimization of 1*H*-indazol-3-amine derivatives as potent Fibroblast Growth Factor Receptor inhibitors

Jing Cui[a,1], Xia Peng[b,1], Dingding Gao[a,1], Yang Dai[b], Jing Ai[b,*], Yingxia Li[a,*]

[a] *School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China*
[b] *Division of Antitumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China.*

ABSTRACT

Fibroblast growth factor receptor (FGFR) is a potential target for cancer therapy because of its critical role in promoting cancer formation and progression. In a continuing effort to improve the cellular activity of hit compound **7r** bearing an indazole scaffold, which was previously discovered by our group, several compounds harnessing fluorine substituents were designed, synthesized and biological evaluated. Besides, the region extended out to the ATP binding pocket toward solvent was also explored. Among them, compound **2a** containing 2,6-difluoro-3-methoxyphenyl residue exhibited the most potent activities (FGFR1: less than 4.1 nM, FGFR2: 2.0±0.8 nM). More importantly, compound **2a** showed an improved antiproliferative effect against KG1 cell lines and SNU16 cell lines with IC_{50} values of 25.3±4.6 nM and 77.4±6.2 nM respectively.

Keywords: FGFR, inhibitors, cellular activity, fluorine.

* Corresponding authors. Tel.: +86 21 50806600x2413 (J.A.), +86 21 51980127(Y.L.).
E-mail addresses: jai@simm.ac.cn, liyx417@fudan.edu.cn.
[1] These authors contributed equally to this work.

The fibroblast growth factor receptor (FGFR) family consists of four RTKs (FGFR1-4) which bind a diverse family of 18 FGF ligands and play a fundamental role in many physiological processes, involving embryogenesis, tissue homeostasis, tissue repair, wound healing, and inflammation.[1,2] In cancer, the constitutive FGFR signaling is activated by gene amplification, point mutations, or chromosomal translocations/rearrangements in several tumor types and involved in cell growth, angiogenesis, cell migration, invasion, and metastasis.[3,4] A recent analysis of 4,853 solid tumors found FGFR aberrations in 7.1% of cancers, gene amplification (66%), mutations (26%) and rearrangements (8%). FGFR1 (mostly amplification) was affected in 3.5% of patients, FGFR2 in 1.5%, FGFR3 in 2.0%, and FGFR4 in 0.5%. The most commonly affected cancers were urothelial (32%), breast (18%), endometrial (~13%), lung (squamous) (~13%), and ovarian (~9%).[5] Owing to their prominent roles in tumor, FGFRs have become crucial targets for cancer therapy.[6-9]

Currently, several second-generation FGFR-selective inhibitors, such as AZD4547, NVP-BGJ398, CH-5183284, and LY-2874455 are under clinical trials targeting patients who have FGFR genetic alterations (Fig. 1).[10-14] As a frontrunner in the group of pan-FGFR inhibitors, AZD4547 was demonstrated to promote favorable therapeutic outcomes against a variety of FGFR-deregulated cancer models, including glioblastoma, non–small cell lung cancer, gastric cancer, and multiple myeloma.[10, 15]

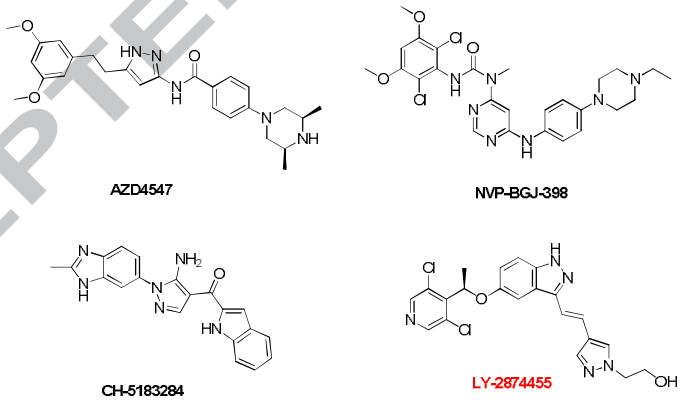


Figure 1. Structures of representative FGFR inhibitors

Based on AZD4547, scaffold hopping and molecular hybridization strategies were utilized to identify the compound **7r** as a potent FGFR inhibitor (Fig. 2). This compound showed potent enzymatic potency against FGFR1 and modest cellular inhibition.[16] Fluorine is often used to improve permeability through the modulation of molecule's lipophilicity, direct fluorine–protein interactions, or reduction of amine basicity. Large amounts of case studies on the use of fluorine interactions in rational drug design have been

reported.[17-20] Herein, we first focus on the incorporation of fluorine substituents on ring A、B and C(**7r**, Fig. 2) to improve the cellular potency(**1a-g** and **2a**) . Then the region extended out to the ATP binding pocket toward solvent was also explored (**2b-g**).

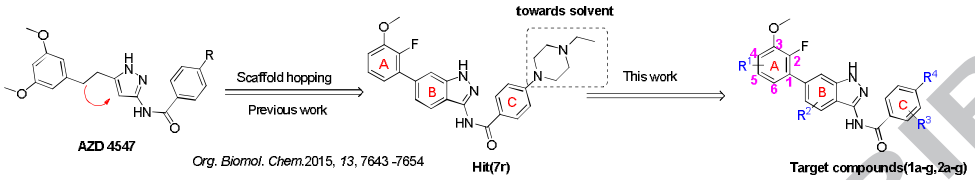
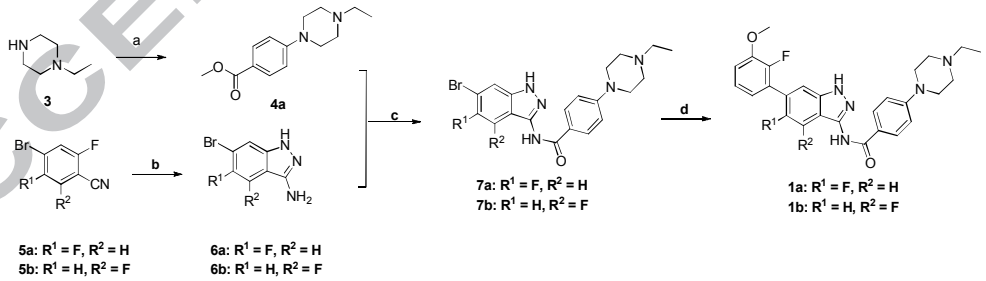
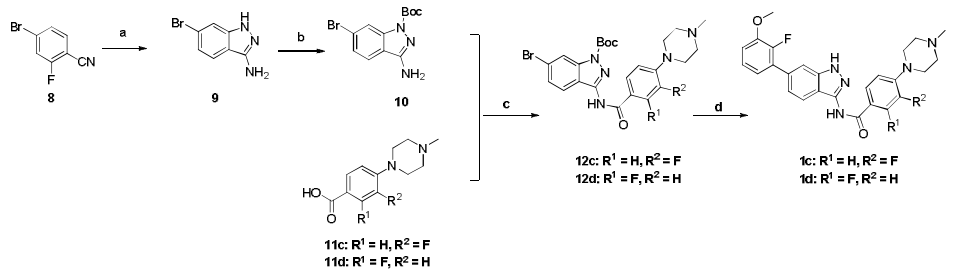


Figure 2. Previous work and design of target compounds

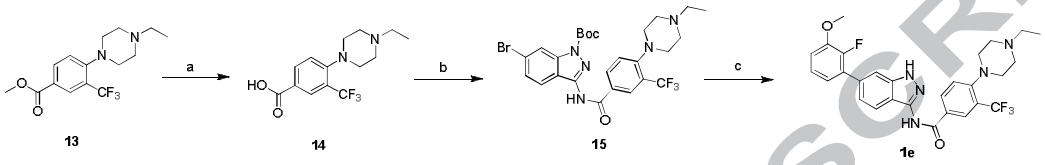
The preparation of target compounds **1a-b** was described in Scheme 1. Compounds **5a-b** were obtained from commercial sources. Coupling of 4-bromo-2-fluorobenzonitrile derivatives with hydrazine gave 6**a-b** in good yields. Then the key intermediates **7a-b** were obtained by condensation of **4a** with **6a-b** in the presence of $Al(CH_3)_3$ at 120°C. Treatment of **7a-b** with 2-fluoro-3-methoxyphenylboronic acid under Suzuki coupling conditions gave target compounds **1a-b**. The preparation of **1c-e** was illustrated in Scheme 2 and 3. Condensation of **11c-d**, **14** with **10** afforded compounds **12c–d** and **15** in the presence of EDCI, DMAP at 60 °C. Finally, coupling of **12c-d** and **15** with 2-fluoro-3-methoxyphenylboronic acid yielded target compounds **1c-e**. The synthesis of compounds **2a-g and 1f-g** was shown in Scheme 4. Condensation of **10** with **4a-g** in the presence of $Al(CH_3)_3$ at 120°C gave intermediates **16a-g**, which directly attempted to Suzuki coupling with 2-fluoro-3-methoxyphenylboronic acid were unsuccessful. After protected the free *NH* of the *1H*-indazol scaffold with Boc group, we successfully got target compounds **2a-g and 1f-g** via Suzuki coupling reaction.



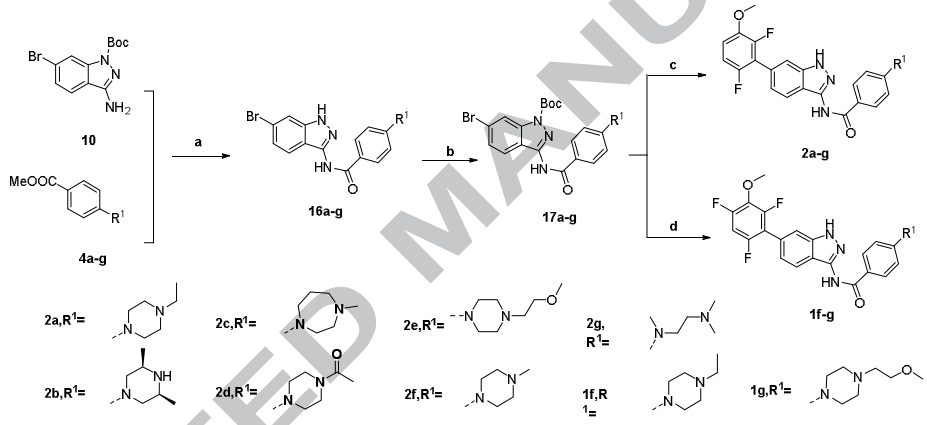
Scheme 1 Reagents and conditions: (a) 4-fuorobenzoate, K_2CO_3, DMSO, 110°C, 89.2%; (b) hydrazine hydrate, *n*-butanol, 120°C, 85.7% (c) $Al(CH_3)_3$, toluene, 120 °C, 15.9% (d) 2-Fluoro-3-methoxyphenylboronic acid, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane, 120 °C, 35.7%-49.6%.



Scheme 2 Reagents and conditions: (a) hydrazine hydrate, *n*-butanol, 120°C, 85.7% (b) Boc_2O, DMAP, THF, 88.7%; (c) EDCI, DMAP, CH_2Cl_2, reflux or HATU, HOAt, DIPEA, CH_2Cl_2, reflux, 10.9%-24% (d) 2-Fluoro-3-methoxyphenylboronic acid, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane, 120 °C, 35.7%-49.6%



Scheme 3 Reagents and conditions: (a) 1M/L NaOH aqueous, CH_3OH, reflux, 100% (b) 9, EDCI, DMAP, CH_2Cl_2, reflux, 2.5% (c) 2-Fluoro-3-methoxyphenylboronic acid, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane, 120 °C, 52.4%.



Scheme 4. Reagents and conditions: (a) $Al(CH_3)_3$, toluene, 120 °C, 16.8%-39.2% (b) Boc_2O, DMAP, THF, 90.2% (c) 2,6-difluoro-3-methoxyphenylboronic acid, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane, 120 °C, 35.7%-49.6% (d)3-Methoxy-2,4,6-trifluorophenylboronic acid, Cs_2CO_3, $Pd(dppf)Cl_2$, dioxane, 120 °C, 35.7%-49.6%.

The target compounds were then evaluated for inhibitory activities against the FGFR1, FGFR2 enzymes and two representative cancer cell lines with different mechanisms of FGFR activation (FGFR1-fusion protein driven human myeloid leukemia cancer cell line KG-1 and FGFR2-amplified dependent human gastric cancer cell line SNU-16). As shown in Table 1, incorporation of fluorine at C-6 position in ring A improved both enzymatic potency and cellular potency (**2a**, FGFR1: less than 4.1 nM, FGFR2: 2.0±0.8 nM, KG1 cell lines: 25.3±4.6 nM, SNU16 cell lines: 77.4±6.2 nM). However, compounds **1a-e** displayed a significant decrease of activities both on enzymes and cells, which indicated that ring B and C were not tolerable to any fluorine substitution patterns. While introducing fluorine at both C-4 and C-6 positions in ring A led to the activity decrease (**1f** and **1g**).

Table 1 *In vitro* enzymatic inhibitory activities and antiproliferative activities of designed compounds

compd.	FGFR1 IC_{50} [a] (nM)	FGFR2 IC_{50} (nM)	KG1 IC_{50} (nM)	SNU16 IC_{50} (nM)
1a	35.2±2.2	9.5±0.1	683.9±114.9	>1000
1b	219.6±104.4	132.7±19.6	>1000 [b]	>1000
1c	147.6±16.0	108.5±11.1	>1000	>1000
1d	>1000	>1000	>1000	>1000
1e	>1000	>1000	>1000	>1000
2a	<4.1	2.0±0.8	25.3±4.6	77.4±6.2
1f	49.0±4.3	35.1±2.4	>1000	>1000
1g	99.3±18.8	91.0±21.6	>1000	>1000
7r	6.2±0.7	4.0±0.4	283.9±13.4	590.8±105.8
AZD4547	1.2±0.1	0.6±0.1	3.3±0.2	3.4±0.2

[a] The IC_{50} values are shown as the mean ± SD (nM) or estimated values from two separate experiments.

[b] If a specific compound is given a value>1000, it indicates that a specific IC_{50} cannot be calculated from the data points collected, meaning‘ no effect ’.

To further improve the antiproliferative activities against KG1 cell lines and SNU16 cell lines, the modification was concentrated on ring C which extended out to the ATP binding pocket toward solvent. Then a diversity of substituents was explored (guided by a calculated lipophilicity threshold). The procedure was shown in Scheme 4 which was similar to the synthesis of compound **2a**. The results were listed in Table 2. In light of these data, all of these tested compounds exhibited potent FGFR1 and FGFR2 enzymatic activities. Actually, we did not found the correlation between clogP and activities in this series compounds. Compared to **7r**, compounds **2a**, **2c** and **2f** not only displayed better enzymatic inhibitory activities, but also exhibited superior antiproliferative activities. However, compounds **2b**, **2d** and **2e** showed weaker cellular activities, perhaps owing to the poor cellular penetration.

Table 2 *In vitro* enzymatic inhibitory activities and antiproliferative activities of designed compounds

compd.	cLog P [b]	FGFR1 IC_{50} [a] (nM)	FGFR2 IC_{50} (nM)	KG1 IC_{50} (nM)	SNU16 IC_{50} (nM)
2a	5.74	<4.1	2.0±0.8	25.3±4.6	77.4±6.2
2b	5.67	14.0±1.5	7.0±0.6	347.7±12.4	582.4±16.3
2c	5.05	3.7±0.3	2.0±0.7	51.5±17.8	101.5±42.9
2d	4.23	13.0±0.2	2.9±1.4	595.4±98.1	322.2±127.0
2e	5.39	10.4±1.4	16.6±2.0	47.3±18.9	430.8±43.1
2f	5.09	5.6±1.5	<4.1	41.5±5.8	35.6±11.5
2g	5.59	21.1±7.6	35.1±2.4	332.6±61.3	365.6±109.5
7r	5.55	6.2±0.7	4.0±0.4	283.9±13.4	590.8±105.8
AZD4547	4.43	1.2±0.1	0.6±0.1	3.3±0.2	3.4±0.2

[a] The IC_{50} values are shown as the mean ± SD (nM) or estimated values from two separate experiments.

[b] The cLog P values were calculated by ACD-Labs (Version 6.0).

To elucidate the binding mode of compound **2a** to the FGFR1 protein (PDB code: 4ZSA),[16] molecular docking was performed using Glide module encoded in Schrodinger, Maestro 10.1 (Fig. 3A). For comparison, the binding mode of hit compound **7r** was also generated (Fig. 3B) and superimposed on that of **2a** (Fig. 3C). As depicted in Figure 3, both compounds could tightly bind to the ATP-binding site of FGFR1. In detail, the 3-aminoindazole fragment occupied the hinge region of the protein via three hydrogen bonds

with Glu562 and Ala564 and the phenyl ring of indazole core interacted with Phe 489 via π-π stacking. Additionally, the fluorine substituted 3-methoxyphenyl moiety located at the hydrophobic pocket. Interestingly, compound **2a** with two fluorine atoms at phenyl ring rendered the orientation greatly changed compared with compound **7r** and gained another hydrogen bond between the methoxy oxygen and the amino group of Asp641. Furthermore, the two fluorine atoms can form hydrophobic interactions with Ala640 and Val492. These favorable changes could be the possible reasons that compound **2a** showed a slightly stronger activity than compound **7r**.

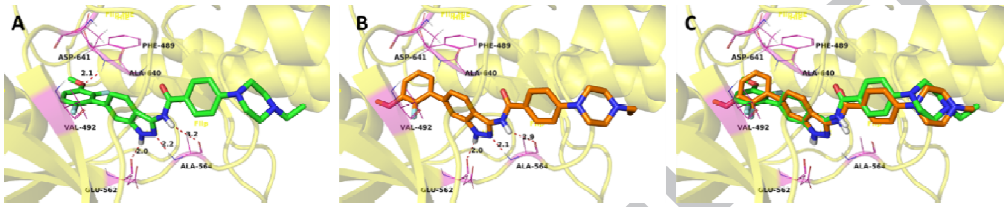


Figure 3. (A) Predicted binding mode for compound **2a** (B) Predicted binding mode for compound **7r** (C) A comparison of the binding mode of **2a** (green) and compound **7r** (orange) with FGFR1. The pictures were generated using Pymol.

In summary, the search to find compounds that exhibited better cell potency than hit **7r** led us to discovery many fluorine substituents with significantly improved cell potency. The basic *N*-ethylpiperazine moiety which extended out to the ATP binding pocket toward solvent was also optimized. As we expected, the most potent compound **2a** exhibited increasing enzymatic and antiproliferative activities (FGFR1: less than 4.1 nM, FGFR2: 2.0±0.8 nM, KG1 cell lines: 25.3±4.6 nM, SNU16 cell lines: 77.4±6.2 nM). Further investigation on the mechanism and in *vivo* antitumor activity of compound **2a** are undergoing and will be reported in due course.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant No. 81473075, 81473243), and "Personalized Medicines–Molecular Signature-based Drug Discovery and Development" and Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA12020103).

Supplementary data

Supplementary data is available on the publishers' web site along with the published article.

References and notes

1. Katoh M. Fgfr Inhibitors: Effects on Cancer Cells, Tumor Microenvironment and Whole-Body Homeostasis. *Int J Mol Med.* 2016; 38: 3-15.

2. Tiong KH, Mah LY, Leong CO. Functional Roles of Fibroblast Growth Factor Receptors (Fgfrs) Signaling in Human Cancers. *Apoptosis,* 2013; 18: 1447-1468.
3. Ahmad I, Iwata T, Leung HY. Mechanisms of Fgfr-Mediated Carcinogenesis. *Biochim Biophys Acta.* 2012; 1823: 850-860.
4. Carneiro BA, Meeks JJ, Kuzel TM, Scaranti M, Abdulkadir S A, Giles FJ. Emerging Therapeutic Targets in Bladder Cancer. *Cancer Treat Rev.* 2015; 41: 170-178.
5. Helsten T, Elkin S, Arthur E, Tomson BN, Carter J, Kurzrock R. The Fgfr Landscape in Cancer: Analysis of 4,853 Tumors by Next-Generation Sequencing. *Clin Cancer Res.* 2016; 22: 259-267.
6. Touat M, Ileana E, Postel-Vinay S, Andre F, Soria JC. Targeting Fgfr Signaling in Cancer. *Clin Cancer Res.* 2015; 21: 2684-2694.
7. Giacomini A, Chiodelli P, Matarazzo S, Rusnati M, Presta M, Ronca R. Blocking the Fgf/Fgfr System as a "Two-Compartment" Antiangiogenic/Antitumor Approach in Cancer Therapy. *Pharmacol Res.* 2016; 107: 172-185.
8. Zhou WY, Zheng H, Du XL, Yang JL. Characterization of Fgfr Signaling Pathway as Therapeutic Targets for Sarcoma Patients. *Cancer Biol Med.* 2016; 13: 260-268.
9. Helsten T, Schwaederle M, Kurzrock R. Fibroblast Growth Factor Receptor Signaling in Hereditary and Neoplastic Disease: Biologic and Clinical Implications. *Cancer Metastasis Rev.* 2015; 34: 479-496.
10. Gavine PR, Mooney L, Kilgour E, Thomas AP, Al-Kadhimi K, Beck S, Rooney C, Coleman T, Baker D, Mellor MJ, Brooks AN, Klinowska T. Azd4547: An Orally Bioavailable, Potent, and Selective Inhibitor of the Fibroblast Growth Factor Receptor Tyrosine Kinase Family. *Cancer Res.* 2012; 72: 2045-2056.
11. Guagnano V, Furet P, Spanka C, Bordas V, Le Douget M, Stamm C, Brueggen J, Jensen MR, Schnell C, Schmid H, Wartmann M, Berghausen J, Drueckes P, Zimmerlin A, Bussiere D, Murray J, Graus-Porta D. Discovery of 3-(2,6-Dichloro-3,5-Dimethoxy-Phenyl)-1-{6-[4-(4-Ethyl-Piperazin-1-Yl)-Phenylamin-O]-Pyrimidin-4-Yl}-1-Methyl -Urea (Nvp-Bgj398), a Potent and Selective Inhibitor of the Fibroblast Growth Factor Receptor Family of Receptor Tyrosine Kinase. *J Med Chem.* 2011; 54: 7066-7083.
12. Nakanishi Y, Akiyama N, Tsukaguchi T, Fujii T, Sakata K, Sase H, Isobe T, Morikami K, Shindoh H, Mio T, Ebiike H, Taka N, Aoki Y, Ishii N. The Fibroblast Growth Factor Receptor Genetic Status as a Potential Predictor of the Sensitivity to Ch5183284/Debio 1347, a Novel Selective Fgfr Inhibitor. *Mol Cancer Ther.* 2014; 13: 2547-2558.
13. Zhao G, Li WY, Chen D, Henry JR, Li HY, Chen Z, Zia-Ebrahimi M, Bloem L, Zhai Y, Huss K, Peng SB, Mc-Cann D J. A Novel, Selective Inhibitor of Fibroblast Growth Factor Receptors That Shows a Potent Broad Spectrum of Antitumor Activity in Several Tumor Xenograft Models. *Mol Cancer Ther.* 2011; 10: 2200-2210.
14. Cheng W, Wang M, Tian X, Zhang X. An Overview of the Binding Models of Fgfr Tyrosine Kinases in Complex with Small Molecule Inhibitors. *Eur J Med Chem.* 2016; 126: 476-490.
15. Zhang J, Zhang L, Su X, Li M, Xie L, Malchers F, Fan S, Yin X, Xu Y, Liu K, Dong Z, Zhu G, Qian Z, Tang L, Schottle J, Zhan P, Ji Q, Kilgour E, Smith PD, Brooks AN, Thomas RK, Gavine PR. Translating the Therapeutic Potential of Azd4547 in Fgfr1-Amplified Non-Small Cell Lung Cancer through the Use of Patient-Derived Tumor Xenograft Models. *Clin Cancer Res*. 2012; 18: 6658-6667.
16. Liu J, Peng X, Dai Y, Zhang W, Ren S, Ai J, Geng M, Li Y. Design, Synthesis and Biological Evaluation of Novel Fgfr Inhibitors Bearing an Indazole Scaffold. *Org. Biomol. Chem.* 2015; 13: 7643-7654.
17. Vulpetti A, Dalvit C. Fluorine Local Environment: From Screening to Drug Design. *Drug Discov Today.* 2012; 17: 890-897.
18. Bauer MR, Jones RN, Baud MG, Wilcken R, Boeckler FM, Fersht AR, Joerger AC, Spencer J. Harnessing Fluorine-Sulfur Contacts and Multipolar Interactions for the Design of P53 Mutant Y220c Rescue Drugs. *ACS Chem Biol.* 2016; 11: 2265-2274.
19. Muller K, Faeh C, Diederich F. Fluorine in Pharmaceuticals: Looking Beyond Intuition. *Sci.* 2007; 317: 1881-1886.

20. Hagmann WK. The Many Roles for Fluorine in Medicinal Chemistry. *J Med Chem.* 2008; 51: 4359-4369.

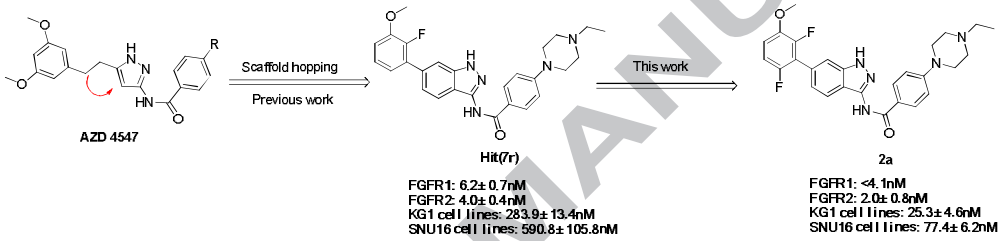
Optimization of 1*H*-indazol-3-amine derivatives as potent Fibroblast Growth Factor Receptor inhibitors

Jing Cui[a,1], Xia Peng[b,1], Dingding Gao[a,1], Yang Dai[b], Jing Ai[b,*], Yingxia Li[a,*]

[a] *School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China*

[b] *Division of Antitumor Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China.*

In a continuing effort to develop more potent cellular activity of hit compound **7r** which was previously discovered by our group, several compounds harnessing fluorine substituents were designed, synthesized and biological evaluated. Additionally, the region extended out to the ATP binding pocket toward solvent was also explored.



* [*] Corresponding authors. Tel.: +86 21 50806600x2413 (J.A.), +86 21 51980127(Y.L.).
* E-mail addresses: jai@simm.ac.cn, liyx417@fudan.edu.cn.
* [1] These authors contributed equally to this work.
*